PROJECT NUMBER: 6908
PROJECT TITLE: Smoke Condensate Studies
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I. TSNA PRECURSORS

A. Objective: To determine the precursors of MS TSNA.

B. Results: Spray application of a protein (1% level), bovine serum albumin (BSA), to oriental base web had no effect on smoke NO or TSNA delivery. Addition to burley base web at 2% actually reduced the delivery of all TSNA by about 30%.

Attempts to confirm the identity of the component identified by GC retention time and TEA response as NNK by preparation of the 2,4-dinitrophenylhydrazine derivative have been unsuccessful. Under the acidic conditions required, NNK and similar model compounds form salts which do not chromatograph via GC or LC. Other approaches will be investigated.

TSNA analyses were performed on partially air-cured (7 weeks) hydroponically grown bottom leaves from two Burley 21 plants. Although the TSNA amounts are not up to normal fully cured burley levels, TSNA are detected at levels of 3-14% relative to normal DBC burley. The fully cured leaves will be analyzed when available.

DBC burley (J7AAM) was exhaustively water-washed to prepare a substrate for salt add-back studies. The resulting filler contained only traces of TSNA.

The absolute minimum detection levels for TSNA by GC/TEA were established via serial dilution of the calibration standard. The limits obtained are: NNN = 28 pg; NAT = 41 pg; and NNK = 103 pg.

C. Plans:

a. Replicate protein (BSA) addition to base web

b. Confirm identity of NNK isolate

c. TSNA analysis of fully cured, hydroponically grown Burley 21 leaves

D. References:

Haut, S. A. Notebook No. 8468, p. 159.

Morgan, W. R. Notebook No. 8506, p. 185.

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II. TSNA REMOVAL STUDIES

A. **Objective**: To explore the possibility of extracting TSNA from stored tobacco using an extraction fluid compatible with current processing.

B. **Results**: Work continued on the development of a convenient, easily maintained HPLC method for determining nicotine and important minor alkaloid in filler. Increasing the length of the cyano column from 15 to 25 cm permits the additional measurement of anatabine, anabasine and nornicotine in the HPLC method for nicotine in filler. Various solvent systems are being investigated to improve filler extraction without interfering with the chromatography. C-18 SPE cartridges are being used to concentrate filler extracts which contain low levels of the minor alkaloids (e.g., from previously extracted tobaccos). At pH 11, the alkaloids are retained; salts are removed with water, and the alkaloids are then eluted with the HPLC solvent.

C. **Plans**: Find a filler extraction solvent that yields adequate alkaloid recoveries and that is compatible with HPLC requirements.

D. **References**:

Warfield, A. H. Notebook No. 8558, p. 8.

III. DECOMPOSITION OF TSNA

A. **Objective**: To evaluate the thermal stability of TSNA and investigate methods to enhance thermally induced decomposition of these compounds.

B. **Results**: Studies of TSNA decomposition in the presence of the antioxidant ascorbyl palmitate (AP) continued. At 300°, 250° and 200°C, NNN decomposition values agreed well with previously obtained values. Values for NNK, however, were not consistent with earlier data. Both the NNN and NNK values were obtained using the same experimental set-up.

C. **Plans**: The NNK experiment will be repeated.

D. **Reference**:

Morgan, W. R. Notebook No. 8506, p. 185.

IV. ALTERNATIVES TO CARBON TRAPPING OF NICOTINE IN SCFE TOBACCO

A. **Objective**: To determine the efficacy of other nicotine trapping systems in the removal of TSNA from the process stream.

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B. **Results**: Analysis of a new stem material being used as the nicotine adsorber in the supercritical fluid extraction of tobacco and analysis of the tobacco indicates that TSNA levels are reduced in the extracted filler.

C. **Plans**: Analyses will continue to be carried out as required.

D. **References**: Haut, S. A. Notebook No. 8468, p. 159.

V. CARBONYL CONTENT OF SMOKE FROM A MODEL CIGARETTE OF LOW BIOLOGICAL ACTIVITY

A. **Objective**: To determine the chemical components responsible for the poor subjectives of smoke from a model cigarette of low biological activity (washed shredded bright stems), and to decrease the levels of these components.

B. **Results**: Last month it was reported that the WSBS cigarette X6D7CEA (whistle-through filter; control) gave an unusually high combined acrolein/acetone peak (1824 ug/cig.), which appeared to be drastically reduced by a THAM (trishydroxymethylaminomethane) P/S/P filter. The control cigarette was re-smoked and analyzed using both the usual acetonitrile/water HPLC gradient, which gives a combined acrolein/acetone peak, and a newly developed methanol/water gradient, which clearly separates these peaks. The values obtained (466 vs. 201 + 255 ug/cig., respectively) are in nominal agreement with other WSBS models. It is quite probable that the earlier high value was an artifact produced when the X6D7CEA control was the first cigarette smoked after the rotating head of the Borgwaldt smoking machine had recently been cleaned with acetone.

Three models of WSBS and two models of FFB cigarettes (with and without THAM filters) were sent to H. Spielberg for limited subjective panel testing.

C. **Plans**: Continue evaluation of various model systems as required.

D. **References**:

Levins, R. J. Notebook No. 8558, p. 47.

V. CROSSED SOLUBLES/BASEWEB STUDY (CHEMISTRY)

A. **Objective**: To investigate the smoke chemistry of model cigarettes made from all possible combinations of solubles from bright, burley, and oriental tobaccos on base webs from the same three tobaccos.

B. **Results**: The dialysis performed last month on CEL sub-fraction BuS1 gave a recovery of less than 13% of the exchanged material. This is very surprising in that a 1,000MW cut-off membrane bag was employed. This work will be continued based on the encouraging

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drop in total salts: BuS1 (control) - 29.5% salt; BuS1 (dialyzed) - 2.50% salt.

The North Panel has begun subjective evaluation of the crossed solubles/BW cigarettes.

NRM (ninhydrin reactive material) and protein values were determined on 26 samples obtained from three different experiments which utilized reducing sugars (glucose and fructose).

C. **Plans**: Continue to modify CEL fractions for RL preparation.

D. **References**:

Williams, D. Notebook No. 8530, p. 22.

VI. SUPPORT FUNCTION; CONDENSATE PREPARATION

A. **Objective**: To fabricate cigarettes, perform smokings, and prepare condensate as needed for biological and chemical analysis.

B. **Results**: The CSC from four specially prepared models of WSBS cigarettes with modified filters was collected and furnished as DMSO solutions for a glutathione depletion assay time study.

C. **References**:

Hellams, R. D. Notebook No. 8480, p. 177.

Lambert, E. A. Notebook No. 8523, p. 49.

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